genetic approaches to study mitochondrial Ca^{2+} regulation in various cell types including cardiac myocytes. Basal tyrosine phosphorylation of MCU was reported in human sample mass spectroscopy, but the post translational modifications of MCU are completely unknown.
Hypothesis: Tyrosine phosphorylation of MCU can modulate the mitochondrial Ca^{2+}-uptake rate in cardiac cells.
Methods: MCU was transiently or stably overexpressed in cardiac H9C2 cells. Tyrosine phosphorylation of MCU was detected by a general anti-phosphotyrosine antibody. Mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$) was measured by mitochondrial matrix-targeted Ca^{2+}-sensitive inverse pericam (Mitycam).
Results: α_1-adrenergic stimulation by phenylephrine enhanced the translocation of a Ca^{2+}-dependent tyrosine kinase named proline-rich tyrosine kinase 2 (Pyk2) from cytosol to mitochondria followed by the increase in mitochondrial Pyk2 activity. Overexpressed MCU was exclusively localized at mitochondria and tyrosine residues in MCU were phosphorylated after Pyk2 activation. In addition, Pyk2 was bound to MCU at the basal condition and this interaction was enhanced by phenylephrine treatment. Moreover, Pyk2-dependent phosphorylation of MCU enhances MCU olgomerization observed by a conventional native PAGE. These effects were abolished by the co-transfection of kinase-dead Pyk2. In MCU-overexpressed cells, $[Ca^{2+}]_m$ increased rapidly and reached to higher levels in response to cytosolic Ca^{2+} transients evoked by thapsigargin compared to non-transfected cells. Moreover, peak $[Ca^{2+}]_m$ in MCU-overexpressed cells reached to much higher levels by phenylephrine pretreatment compared to non-treated cells.
Conclusion: α_1-adrenergic stimulation accelerates mitochondrial Ca^{2+} uptake through Pyk2-dependent direct phospholylation of MCU, which promotes the formation of tetrametric MCU channel pore. Our findings open up an exciting opportunity for investigating the first candidate cell signaling pathway for the MCU post translational modifications in cardiac cells.

3378-Pos Board B533
Reverse-Mode of the Mitochondrial Transhydrogenase Consumes NADPH and Provokes Oxidative Stress in Response to Elevated Cardiac Workload
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Mitochondrial production of reactive oxygen species (ROS) contributes to the progression of heart failure, but the mechanisms of ROS generation are incompletely resolved. Superoxide ($^{\cdot}O_2^-$) is generated at the electron transport chain, dismutated to H_2O_2 and eliminated by enzymes that require NADPH. The nicotinamide nucleotide transhydrogenase (NNT) is highly expressed in the heart and catalyzes the reaction NADH+$NADP^+$ to NADPH+NAD^+. Since this reaction is coupled to the proton-motive force, it is perceived that the NNT prevents ROS production by regenerating mitochondrial NADPH. The exact role of the NNT in cardiomyocyte biology, however, has never been assessed. We took advantage of a loss-of-function mutation in the Nnt gene in C57BL/6J, but not C57BL/6N mice. In isolated cardiac myocytes exposed to a physiological increase in workload, β-adrenergic stimulation led to mitochondrial Ca^{2+}-induced Krebs cycle activation with NADH regeneration, providing a substrate for the forward mode NNT reaction to regenerate NADPH. In contrast, under Ca^{2+}-free conditions in isolated mitochondria, acceleration of NADH-coupled respiration by ADP favoured the reverse-mode NNT reaction, regenerating NADH by oxidizing NADPH. Accordingly, in response to an elevated workload in isolated hearts, the NADPH-coupled antioxidants glutathione and peroxiredoxin were oxidized through reverse-mode NNT reaction. This resulted in elevated mitochondrial formation of H_2O_2 *in vivo* after thoracoaortic constriction (TAC) for 6 weeks. In NNT-deficient C57BL/6J mice, TAC-induced oxidative stress *in vivo*, cardiac fibrosis, left ventricular dysfunction and early mortality were ameliorated. Furthermore, scavenging mitochondrial ROS with the peptide SS-31 *in vivo* reduced TAC-induced mortality in C57BL/6N mice to levels observed in C57BL/6J mice. In conclusion, we believe that we discovered the mechanism how an inadequate increase in cardiac workload produces mitochondrial oxidative stress that leads to maladaptive cardiac remodelling and cardiac decompensation.

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Mitochondrial Dysfunction Accompanied by ERK-Dependent Phosphorylation of TFAM in a Chronic MPP+ Model
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Mitochondrial transcription factor A (TFAM) plays pivotal roles in packaging mitochondrial DNA (mtDNA) and regulating its transcription in mammalian mitochondria. We have previously shown that chronic stress induced by repeated low-dose applications of the complex I inhibitor 1-methyl-4-phenylpyridinium (MPP+) results in impaired biosynthesis of mtDNA-encoded proteins, accompanied by a reduction in TFAM expression. MPP+ - induced mitochondrial dysfunction and TFAM down-regulation was markedly inhibited by U0126, ERK1/2 RNAi or transfection of dominant-negative MEK1. Here, we report that TFAM is post translationally modified by phosphorylation during chronic MPP+ treatment through a mechanism reversed by U0126. In addition, we demonstrate that TFAM is a direct target of ERK2 as assessed in an *in vitro* kinase reaction, and are characterizing potential phosphorylation sites. Intriguingly, we also observed reduced levels of TFAM in midbrain tissue from a transgenic mouse model of autosomal dominant Parkinson's disease; 2-D immunoblot analysis suggests increased phosphorylation of both TFAM and ERK1/2, suggesting the possibility of common mechanisms in the toxin and genetic models. Further investigations of the biological significance of TFAM phosphorylation may shed light on mechanisms regulating mitochondrial homeostasis and cell fate in response to disease-related cellular stresses.

3380-Pos Board B535
Properties of the Mitochondrial Permeability Transition in Drosophila S2R+ Cells
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The mitochondrial permeability transition, caused by factors such as reactive oxygen species or calcium overload, has been extensively studied in mammalian cells, but its presence remains in doubt in non-mammalian organisms. In Drosophila, prior studies have documented calcium-induced depolarization and release, but no obvious swelling. Here we show that Drosophila S2R+ cells do possess the machinery for permeability transition, but that its requirement for calcium overload is significantly higher than in mammalian systems. using a calcein-loading method, we show that Drosophila permeability transition can be triggered by calcium overload, using ionomycin, and by cysteine oxidation, using phenylarsine oxide. As in mammalian systems, pharmacological blockade of mitochondrial cyclophilin (cyclosporine A) or the ATP/ADP transporter (bongkrekic acid) inhibits the Drosophila permeability transition. Finally, we examine the pathways for calcium influx into S2R+ mitochondria to see if differences in these pathways between mammalian and Drosophila cells may partly explain the discrepancy in calcium requirement.

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Mitochondrial Potassium Channels in Dictyostelium Discoideum
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Mitochondria are crucial not only in energy metabolism but also in regulation of cell senescence and apoptosis. The strict control of inner mitochondrial membrane permeability and selective ion transport is essential for mitochondria functioning. Potassium ions homeostasis is an important process for mitochondrial optimal functioning. Potassium channels such as ATP-regulated, large conductance calcium activated and voltage dependent channels were observed in inner mitochondrial membrane in various mammalian tissues. Recently, we have identified potassium channels in inner mitochondrial membrane of potato *Solanum tuberosum* and *Acanthamoeba castellanii*. Currently we characterize mitochondrial potassium channels from one of Dictyostelium species. It is commonly used as a model organism to study cell differentiation, metabolism and programmed cell death. Preliminary experiments are focused on biophysical and pharmacological characterization of mitochondrial ion channels. Purified inner mitochondrial membranes (submitochondrial particles) were reconstituted into planar lipid bilayer. To form model membranes asolectin from soybean mixture of phospholipids was used. We observed two types of potassium selective ion channels in submitochondrial particle samples: a large- and small-conductance channels. Experiments were performed both in gradient solution 50/150 mM KCl (cis-trans) and in symmetrical solution 150/150 mM KCl at voltages from −50 to 50 mV. Regulation of the channel activity by divalent cations such as Ca^{2+} and Mg^{2+} was explored. Additionally, interaction of the ATP with mitochondrial potassium channels was characterized. The knowledge on mitochondrial ion channels may contribute to understanding molecular mechanism of *Dictyostelium discoideum* functioning.
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